THE OSMOTIC PROPERTIES OF LIVING CELLS (EGGS OF ARBACIA PUNCTULATA)

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When a cell such as the unfertilized egg of the sea urchin *Arbacia punctulata* is placed in a solution hypotonic with respect to its natural medium, water enters and the cell swells; conversely, it shrinks in hypertonic solutions. This observation at once suggests that the force which causes water to enter is osmotic pressure, that the cell is acting as an osmometer. Earlier measurements of cells in equilibrium with anisotonic solutions indicated that the unfertilized *Arbacia* egg is an osmometer of a high degree of excellence, although the observed volume of cells at equilibrium fell somewhat short of the calculated value (1). Since the relations between pressure and volume of the cell forms the basis for a kinetic theory of osmosis which will be given in a subsequent paper (2), it was necessary to determine this relation as accurately as possible, by means of further experiments.

The present paper therefore attempts to answer the question: how nearly ideal in its osmotic properties is the *Arbacia* egg?

An ideal osmometer should meet the following requirements: (1) One should be able to measure volume accurately at all pressures. (2) The product of pressure and volume should be constant (law of Boyle-Van't Hoff). (3) The membrane should be actually semipermeable, *i.e.*, should allow passage of water, but not of dissolved substances. (4) The transport of water should be due to osmotic force alone.

These requirements will be taken up in order.

1. Measurement of Cell Volume

One of the greatest advantages of the *Arbacia* egg, as compared with other cells used as natural osmometers, is its generally spherical

shape. This fact, together with its relatively large size, allows direct and accurate measurement of diameter (and so of volume) with microscope and ocular micrometer.

In measuring, only optically round cells—these constitute the great majority—are selected. When these are rolled over under the microscope they are seen to be spherical in fact.

However there remains the possibility that the eggs might flatten under their own weight as they rest on the bottom of the dish.* If such flattening take place, the measurement of the horizontal optical section would cause the volume to be overestimated, and our data would need to be corrected.

The simultaneous measurement of horizontal and vertical diameters of an egg should present no serious difficulties. However, we have employed an even simpler method for detecting the flattening of eggs under their own weight. This method consists in placing a sample of eggs in a water-tight chamber, whose top and bottom are formed of cover-slips. After settling, the diameters of 50 eggs are measured, in the ordinary way. The chamber is now gently turned over; with the exception of a very small percentage the eggs will stick to the surface on which they were originally resting, and so come to hang from what is now the top of the chamber. The same force which tended to flatten the eggs as they rested on the bottom now tends to elongate them. The horizontal diameters of approximately the same 50 eggs may again be measured; if there should be any tendency for the eggs to become deformed under the action of their weight, it would be detected in a diminution of the diameter of the optical section. Moreover, the effect would be (approximately) doubled.

In order to facilitate the sticking of the eggs it is advisable first to coat the cover-slips with a thin film of albumin—especially when eggs are in equilibrium with the more hypotonic solutions, as in these solutions the natural stickiness of the eggs is considerably diminished.

Table I gives the results of several such experiments. The average volume of 50 cells is used in each case; in the first column is the concentration of sea water in which the eggs are in equilibrium; in the second column is the average volume computed from measurements

* Vlés (3) has reported that flattening occurs in the eggs of another species of sea urchin.

made with the eggs resting on the bottom of the chamber; in the last column is the average volume of approximately the same 50 eggs, computed from measurements of diameter made with the chamber inverted and the eggs hanging from the top.

It is seen that the volumes of resting and hanging eggs agree very closely. We conclude that in these experiments there is no deformation of the eggs, under the action of their weight.

There remains the possibility of a semipermanent deformation, which requires an appreciable time to become established. In that

TABLE I

Experiment to determine whether cells are flattened. In Column 1 is given the concentration of sea water with which cells are in equilibrium. Columns 2 and 3 give volume of cells resting on the bottom of the chamber and suspended from the top, respectively.

Each figure represents the mean of 50 cells. These figures must be multiplied by 100 to obtain actual volumes in cubic micra.

The figures indicate that these cells are not appreciably flattened either in isotonic or hypotonic sea water.

Concentration of sea water	Cell volumes	
	Bottom of chamber	Top of chamber
per cent		
100	2015	2015
60	3184	3184
60	3162	3195
50	3900	3924
50	3814	3850

case our measurements would fail to detect the deformation, as they are made within a few minutes of inverting the cell. However, such a semipermanent deformation could readily be detected by rolling the eggs over, under observation. Measurements made in this way on a number of diameters of the egg have failed to show any appreciable lack of sphericity.

We may thus conclude that the eggs of this species are not appreciably deformed in any of the solutions used in these experiments, under the action of their weight in these media.

2. *Application of the Boyle-Van't Hoff Law*

In the theory of the ideal, dilute solution, the osmotic pressure of a given amount of dissolved substance is inversely proportional to the volume in which it is distributed. The assumption that the solutions of the present system behave as ideal, dilute ones is an approximation which permits the application of the simple laws governing osmotic processes. An attempt to evaluate the activities of all the constituents of the cell and its medium is of course out of the question.

We may, therefore, write,

$$PV = K \tag{I}$$

where P is the osmotic pressure of the dissolved substances in the egg, V is the volume of the egg, and K is a constant. From the consideration that the egg initially must be in equilibrium with ordinary sea water, of known osmotic pressure, it is seen that K may be determined experimentally by measuring the volume of the egg as it rests in its normal medium. We write for (I) its equivalent:

$$PV = P_o V_o \tag{I a}$$

where P_o is the osmotic pressure of ordinary sea water and V_o the volume of the egg in its normal medium.

This equation furnishes a method for determining, at any time, the osmotic pressure in the interior of the egg. In addition, it furnishes the law that the equilibrium volumes of the egg must follow in anisotonic solutions. In these cases the internal pressure must equal that of the external medium, and the expected volume of the egg at equilibrium is given by

$$P_{ex} V_e = P_o V_o \tag{I b}$$

where P_{ex} is the osmotic pressure of the external medium (known from its concentration) and V_e is the expected equilibrium volume of the egg. (I *b*) therefore furnishes the means of determining whether the Boyle-Van't Hoff law is obeyed by this osmometer.

The earliest measurements of the *Arbacia* egg in equilibrium with hypotonic solutions were made by R. S. Lillie (4). He found that when sea water was diluted two and one-half times the eggs only doubled

in volume. As stated above smaller discrepancies were reported by the present authors in an earlier paper (1).

Experimental Method

Eggs from a single animal were washed and very gently centrifuged. The supernatant fluid was discarded. The remaining heavy suspension of eggs was distributed in 200 cc. of the several dilutions of sea water. Subsequently, the eggs were pipetted into fresh solution contained in Stender dishes. These were kept at room temperature 19°C. Measurements were made from 2 to 5 hours later, after repeated preliminary measurements showed that the cells had reached constant volume.

In two subsequent experiments the cells were stored over night at 10°C. and measured the following morning. Measurements were made in one case at room temperature, in the other at 10 to 12°C.

The optical system employed was a 10 mm. objective (immersed in the sea water) and a filar ocular micrometer, giving a magnification of 240 diameters.

The solutions employed were undiluted sea water (100 per cent), 90 per cent (*i.e.*, 90 parts of sea water with 10 parts of distilled water) 80, 70, 60 and 50 per cent. 50 cells in equilibrium with each of these solutions were measured by each of two observers.

After measurement, cells (in two of the experiments) were returned to ordinary sea water and sperm was added. Normal cleavage took place, except in the case of cells taken from 50 per cent sea water; these showed atypical cleavage.

RESULTS

The results of three experiments are recorded in Table II.* In the first column are given relative pressures, in the second observed volumes and in the fifth column, volumes calculated by Equation (I *b*). The combined data are presented graphically in Fig. 1, where the lower curve represents observed volumes of 300 cells plotted against the relative pressure (or concentration) of the outside solution, and the upper curve is calculated by Equation (I *b*). It is seen that there is a slight deviation of the two curves which becomes more marked as we pass from higher to lower pressures. But even in 50 per cent solution the cells fail by less than 6 per cent to attain the volume calculated. Therefore Equation (I *b*) proves to be a rather fair first approximation.

* The three experiments reported in this paper are selected as probably the most accurate of a large number of determinations made over a period of several years.

TABLE II

Summary of three equilibrium experiments. In the first column the osmotic pressure of the solutions is given as a fraction of the pressure of ordinary (100 per cent) sea water, which is taken as unity. Each observed volume in Column 2 represents the mean of 100 cells. These figures must be multiplied by 100 to obtain the actual volume in cubic micra.

The remaining columns are calculated from the first and second columns. Column 3 is their product; the values show a drift. Column 4 is their product after volumes have been corrected for osmotically inactive material, "*b*"; these values do not show a drift. Columns 5 and 6 have been computed by means of Equations (I *b*) and (II *b*) respectively. In the former, volumes diverge from those of Column 2 (observed values) whereas in Column 6, volumes are in good agreement with those of Column 2.

We have determined the probable error of mean diameters corresponding to the mean observed volumes as given in Column 2. In each case the probable error approximates ± 0.1 micron, and ranges from ± 0.07 to ± 0.15 micra. For example the diameter corresponding to the first volume in Column 2 is 74.10 ± 0.08 micra. From this it is seen that ova from any one animal are remarkably uniform in size.

Experiment	(1) Relative pressure	(2) Volume observed	(3) PV	(4) $P(V-b)$	(5) Volume calculated from $V_e = \frac{V_o P_o}{P_{ex}}$	(6) Volume calculated from $V_e = \frac{(V_o-b)P_o}{P_{ex}} + b$
				(b=150)		
A	1.0	2130	2130	1980	2130	2130
	0.9	2352	2117	1982	2367	2350
	0.8	2612	2090	1970	2663	2625
	0.7	2975	2083	1978	3043	2979
	0.6	3471	2083	1993	3550	3450
	0.5	4125	2063	1988	4260	4110
				(b=250)		
B	1.0	1989	1989	1739	1989	1989
	0.9	2167	1950	1725	2210	2182
	0.8	2407	1926	1726	2486	2424
	0.7	2727	1909	1734	2841	2734
	0.6	3194	1916	1766	3315	3148
	0.5	3738	1869	1744	3978	3728
				(b=310)		
C	1.0	2245	2245	1935	2245	2245
	0.9	2428	2185	1906	2494	2460
	0.8	2691	2153	1905	2806	2729
	0.7	3063	2144	1927	3207	3074
	0.6	3596	2158	1972	3742	3535
	0.5	4144	2072	1917	4490	4180

The discrepancy, however, is a real one. In Column 3 of Table II are given the pressure volume products of each experiment. In each case it is seen that there is a slight but definite drift in values of PV.

There are a number of factors which might prevent the cell from swelling as much as predicted. Of these the one that is surely important and which might account for practically the entire discrepancy is that obviously a considerable fraction of the cell volume is occupied

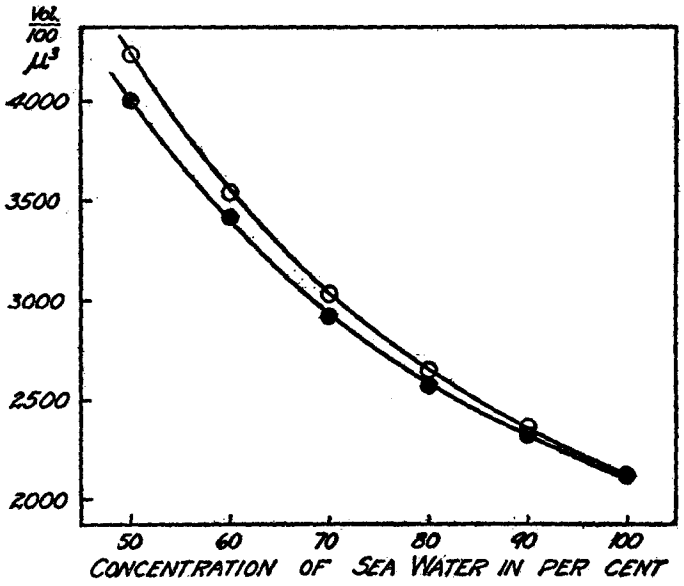

FIG. 1. Comparison of observed and calculated volumes. Solid circles represent mean volumes of 300 cells in osmotic equilibrium with various dilutions of sea water. Open circles are volumes calculated by Equation (I*b*). Data are taken from Table II.

by material that can exert little or no osmotic pressure. This fact is best demonstrated by rapidly centrifuging the eggs, whereupon zones of oil and pigment granules separate from the rest of the cytoplasm. The osmotically active solution therefore occupies a volume which is equal not to V but to $V - b$, in which b is the volume occupied by osmotically inactive material.

Equations (I) to (I b) now become, respectively,

$$P(V - b) = K \quad \text{(II)}$$
$$P(V - b) = P_o(V_o - b) \quad \text{(II } a\text{)}$$
$$P_{ex}(Ve - b) = P_o(V_o - b) \quad \text{(II } b\text{)}$$

The value of b may be found by substituting in Equation (II b) the values given in Columns 1 and 2 of Table II. Since this method of computing gives a considerable scattering of values for b, a graphic method is preferred, one in which all points can be weighed equally. Observed volumes are plotted against the reciprocal of the relative pressures, a straight line is fitted to the points and extrapolated to $\frac{1}{P} = 0$, and the corresponding volume is read off. In these three experiments, b is found to equal 150, 250 and 310 respectively; for the combined data, b equals 240, which is 11 per cent of the cell volume in ordinary sea water. This computation therefore indicates that osmotically inactive material occupies in the neighborhood of 11 per cent of the cell volume.*

In Column 4 of Table II, $P(V - b)$ has been calculated for these experiments. The resulting values show no definite drift, so that it may be stated that Equation (II) fits the data satisfactorily. The last column of Table II gives volumes calculated by Equation (II b). It is seen that these values are in good agreement with observed volumes (Column 2).

These experiments, therefore, show that with this material the Boyle-Van't Hoff law, corrected for volume occupied by osmotically inactive material, holds for pressures of 11 to 22 atmospheres.

It is interesting to compare these conclusions with those of other workers based on different types of isolated cells.

Höfler (5), with certain plant cells containing a large sap vacuole surrounded by an extremely thin layer of protoplasm, found that the pressure-volume product over a certain range of osmotic pressures is constant; that is, b, as defined above, is equal to zero.

Ege (6) found that the "disperse phase," b, in rabbit erythrocytes is equal to about 40 per cent of the cell volume, while Wieringa (7) reported that in certain yeasts, the volume occupied by osmotically

* b so determined also includes volume of solutes themselves.

inactive material plus the volume of the cell wall equals about 64 per cent of the cell volume.

Making corrections for these values of "*b*," both Ege and Wieringa found close agreement between calculated and observed volumes of cells.

3. The Semipermeability of the Membrane

Of the other factors that might prevent the cell from attaining the equilibrium volume predicted by Equation (I *b*), imperfect semipermeability of the cell membrane at once comes to mind.* Leakage of salts or other contents from the cell in hypotonic solutions would of course have this effect.

However, it is improbable that such leakage occurs to an appreciable extent in uninjured cells. As shown above, the differences between volumes observed and those calculated by Equation (I *b*) is slight and is satisfactorily accounted for by the presence of osmotically inactive material.

Another type of experiment supports the view that the semipermeability of the membrane is practically perfect. Cells are measured in ordinary sea water, then swollen in a hypotonic solution, returned to ordinary sea water and remeasured after they have come into equilibrium. If cell contents escaped during swelling, it is unlikely (though not impossible) that the first and second measurements should agree. But early experiments (8) indicated that this is the case, that swelling is completely reversible.

Since the question seemed important the experiment was carefully repeated last summer.

In Table III it is seen that the original and final volumes are in good agreement. Thus in the first experiment the original mean volume was $1864 \times 10^2\ \mu^3$; after swelling for 1 hour in 50 per cent sea water and shrinking in ordinary sea water the mean volume was $1853 \times 10^2\ \mu^3$. Cells from the same animal swollen in 60 per cent sea water later returned in ordinary sea water to a volume of $1874 \times 10^2\ \mu^3$. Similar results were obtained in several other experiments, of which two are included in Table III.

* It is assumed that the cell surface acts as the membrane of the osmometer.

Thus the weight of evidence is against appreciable leaking of cell contents when the cell is placed in hypotonic solutions, at least over the range of pressures here used. This is true, however, only as long as the cell is uninjured. Injured cells, as has been shown elsewhere (9), lose their semipermeability to a greater or less extent; if loss of semipermeability is marked they behave in hypotonic solutions quite differently from normal cells. In the experiments recorded in Table II, the cells were shown by the fertilization test not to have been injured, except those in 50 per cent solution; these underwent atypical cleavage and therefore were slightly injured.

TABLE III

In these three experiments cells were measured in ordinary sea water (Column 1) and then swollen in 50 per cent sea water for 1 hour. Measurement then showed that they were nearly in equilibrium with this solution. They were returned to ordinary sea water and allowed to shrink for several hours, then remeasured (Column 2).

Similarly cells were swollen in 60 per cent sea water for 1½ hours, then shrunk and remeasured (Column 3).

It is seen that the cells returned approximately to their original volume.

Each figure represents the mean volume of 50 cells. The temperature was 15°C.

Original volume	Final volume in ordinary sea water after swelling in:	
	50 per cent	60 per cent
1864	1853	1874
2020	1994	2027
1823	1882	1800

4. The Effect of Other Forces on the Equilibrium Volume

That forces other than osmotic force affect the volume of the cell in equilibrium with its medium, is highly probable. Elasticity and surface tension at once come to mind. But the fact that Equation (II) fits the data indicates that the magnitude of such forces, compared with that of the osmotic driving force, must be quite small.

SUMMARY

We have attempted to answer the question: How nearly ideal, as an osmometer, is the unfertilized *Arbacia* egg? The following conclusions have been reached:

1. Volumes can be measured accurately over a wide range of pressures since the cell is in general spherical and does not suffer deformation from its own weight or other factors.

2. The product of volume and pressure is approximately constant, if allowance be made for osmotically inactive cell contents. It is computed that from 7 to 14 per cent of cell volume is occupied by osmotically inactive material.

3. Evidence is presented that no appreciable escape of cell contents occurs while the cell is in hypotonic sea water; that, therefore, the semipermeability of the membrane is approximately perfect, so long as injury to the cell is avoided.

4. In comparison with osmotic pressure the influence of other forces, such as elasticity or surface tension, on cell volume must in these experiments be slight.

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